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REVIEW

Serotonin, serotonin receptors and their actions in insects

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Serotonin is an ancient monoamine neurotransmitter, biochemically derived from tryptophan. It is most abundant in the gastrointestinal tract, but is also present throughout the rest of the body of animals and can even be found in plants and fungi. Serotonin is especially famous for its contributions to feelings of well-being and happiness. More specifically it is involved in learning and memory processes and is hence crucial for certain behaviors throughout the animal kingdom. This brief review will focus on the metabolism, biological role and mode-of-action of serotonin in insects. First, some general aspects of biosynthesis and break-down of serotonin in insects will be discussed, followed by an overview of the functions of serotonin, serotonin receptors and their pharmacology. Throughout this review comparisons are made with the vertebrate serotonergic system. Last but not least, possible applications of pharmacological adjustments of serotonin signaling in insects are discussed.

Keywords: behavior; biogenic amine; drug; G protein-coupled receptor; insecticide; learning and memory

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Introduction

Serotonin (5-hydroxytryptamine or 5-HT) is an ancient monoamine neurotransmitter that belongs to the biogenic amines. Biogenic amines are derived from aromatic amino acids and function as neurotransmitters and neuromodulators in the central nervous system or can be released in circulation to act as (neuro)hormones. In vertebrates there are five established biogenic amine neuromodulators: the indolamine 5-HT, the imidazolamine histamine and the catecholamines dopamine, epinephrine (also called adrenaline) and norepinephrine (also called noradrenaline). These biogenic amines are often grouped with other small compounds, such as acetylcholine, glutamate and gamma-aminobutyric acid, as small molecule neurotransmitters. The phenolamines tyramine and octopamine are generally considered as the protostome analogs of the adrenergic amines in vertebrates and other deuterostomes [1-4]. However, octopaminergic systems have also been reported in some deuterostomian phyla (in species, such as the echinoderm sunflower star *Psyconopodia helanthoides* and the cephalochordate *Branchiostoma lanceolatum*) and monophenolic amines are likely to occur in considerable quantities in all triblastic animals, except vertebrates [2,5,6]. In vertebrates, octopamine and tyramine are present in only very small quantities (trace amines) [7].

Biosynthesis of 5-HT

Biogenic amines are synthesized starting from amino acids by only one or a few enzymatic steps, including a decarboxylation. 5-HT belongs to the class of monoamines, where a basic amine group is separated from an aromatic core by an aliphatic chain with two carbon oxides. The synthesis of 5-HT begins with the essential amino acid tryptophan. The enzyme tryptophan hydroxylase (TPH), also known as tryptophan-5-monooxygenase, first adds a hydroxyl group to tryptophan forming 5-hydroxytryptohan (5-HTP) [8] (Figure 1). The hydroxylase TPH constitutes together with phenylalanine hydroxylase (which catalyzes

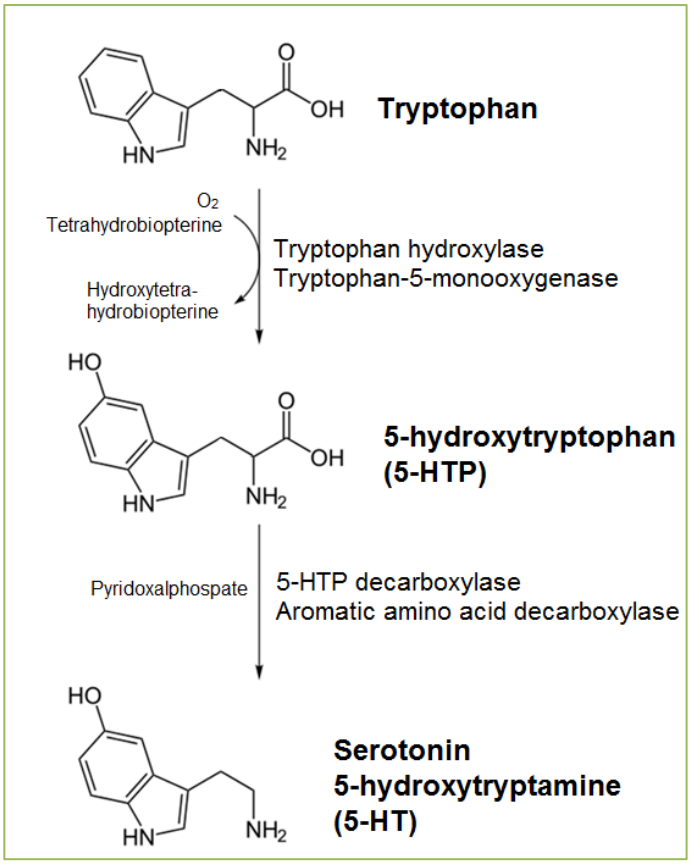

Figure 1. Biosynthesis of serotonin (5-HT). Tryptophan is converted into 5-hydroxytryptamine (5-HTP) by tryptophan hydroxylase (TPH), Next, 5-HTP is converted to 5-HT by 5-HTP decarboxylase (also called aromatic amino acid decarboxylase or AADC).

the hydroxylation of the aromatic side-chain of phenylalanine to generate tyrosine) and tyrosine hydroxylase (which converts tyrosine to 3,4-dihydroxyphenylalanine or DOPA) the non-heme iron and tetrahydrobiopterin-dependent aromatic amino acid hydroxylase family. These enzymes use (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin and O_2 as cofactors [9,10].

In a second step of 5-HT biosynthesis, 5-HTP decarboxylase catalyzes the conversion of 5-HTP to 5-HT [11] (Figure 1). It has been shown that 5-HTP decarboxylase is the same enzyme as dopamine decarboxylase, which catalyzes the decarboxylation step in dopamine biosynthesis, and is generally called aromatic amino acid decarboxylase [12].

Metabolic pathways of 5-HT

To act in the nervous system, biogenic amines are stored at synaptic terminals for release into the chemical synapse in response to neuronal depolarization. In the synaptic cleft, the amines bind specific receptor proteins (see § 5.), which can be present on both pre- and postsynaptic cells. Synaptic effects of the biogenic amines are terminated via re-uptake by binding to monoamine transporters [13-15]. These transporter proteins belong to a subclass of the neurotransmitter/sodium symporter family, a group of glycoproteins characterized by their 12 transmembrane segments (TM) structure [14,16]. The first identified insect amine transporter was the SERotonin Transporter (SERT) from the fruit fly *Drosophila melanogaster* [17,18]. Later, insect transporters with high affinity for dopamine [19] and tyramine/octopamine [13,20-22] were also identified. In humans, these transporters attained much interest because psychoactive compounds both for medication and illegal drugs interact with them [23].

After re-uptake, biogenic amines are repacked into vesicles for re-release or enzymatically inactivated. In vertebrates, the primary catabolic pathway for biogenic amines is oxidative deamination by monoamine oxidases [14]. Little or no monoamine oxidase activity could be measured in insect nervous tissue [24]. Enzymatic inactivation in invertebrates is considered to take place after amino-terminal tagging with specific groups, mainly N-acetylation or N-methylation. However γ-glutamylation, sulphation, and β-alanyl conjugation are also possible metabolic routes [25,26].

Functions of 5-HT in insects

5-HT is a intracellular signaling molecule found in all organisms with a central nervous system. The discovery of 5-HT as an important neuromodulator involved a multidisciplinary approach of several research groups. In the 1940s, during studies of constricting factors in the blood causing hypertension, a serum substance affecting vascular tone was isolated and named “serotonin” [27,28]. A chemical structure elucidation approach (using ultraviolet absorption spectrometry and colorimetric chemical reactions) showed that serotonin was 5-hydroxytryptamine (5-HT) [29]. In 1952, 5-HT was shown to be the same substance as enteramine, a compound deduced in the 1930’s from an extract of the enterochromaffin cells in gastrointestinal mucosa, which caused contraction of the smooth muscle of the rat uterus [30,31]. Approximately 80-90% of the human body’s total 5-HT is located in the enterochromaffin cells of the gut, where it is used to regulate intestinal motility [32,33]. A few years later, 5-HT was proven to be present in mammalian brain, the first step to its recognition as a neuromodulator [34]. The observation that the hallucinatory drug lysergide acid diethylamide (LSD) antagonized a response produced by 5-HT further confirmed the idea that 5-HT may be important for the behaviour [35].

Nowadays, the role of 5-HT is established in the regulation of many important human processes, such as appetite, mood and emotion, sleep, sexual activity, pain, and learning abilities. In different insect species, comparable processes were demonstrated to be affected by 5-HT. For example, in *D. melanogaster*, elevating 5-HT levels, either by treatment with 5-HTP or by overexpressing TPH, significantly increased periods of sleep [36]. On the contrary, entrainment of the *Drosophila* circadian clock to light was inhibited by 5-HT [37]. In several insect species, 5-HT was demonstrated to modulate appetite. In *D. melanogaster*, neuromodulatory actions of 5-HT were shown to depress feeding, while decreased neuronal 5-HT levels increased appetite [38]. In another dipteran species, the flesh fly *Neobellieria bullata*, 5-HT injection in the hemolymph decreased feeding [39]. In the honey bee, 5-HT inhibits feeding when injected in the brain and excites muscle contractions when injected in the gut, although general elevation of 5-HT in the bee's hemolymph did not affect food intake [40]. Consistent with these findings, a 5-HT receptor was identified as a feeding modulation target from a small molecule drug screening in *Drosophila* [41]. In the yellow-fever mosquito *Aedes aegypti*, 5-HT immunoreactive axons surround parts of the salivary gland of adult females [42] and the blood-feeding success was reduced by administration of the 5-HT depleting drug α-methyl-tryptophan [43]. Moreover, 5-HT increased fluid secretion from salivary glands in *A. aegypti*, the fly *Calliphora vicina* and the cockroach *Periplaneta americana* [42,44,45]. In *Rhodnius prolixus*, 5-HT acts as a diuretic hormone. It is produced soon after the initiation of feeding and stimulates rapid tubule secretion [46,47]. In *Drosophila*, where insulin-like peptides both regulate blood sugar and act as growth factors, serotonergic neurons control the adult body size by affecting insulin-like peptide secretion [48,49]. How much food an animal gets is not only dependent on the abundance of food in the environment but also on the animal's social rank, since the stronger or more dominant animals may steal food from the weaker or less dominant ones. This may explain why 5-HT is not only engaged in the recognition of food availability but also of social rank.

5-HT also has thorough effects on some aspects of learning and memory and is thus a major player in modulating several insect behaviors. By inhibiting the serotonergic system in neurons of *D. melanogaster*, appetitive olfactory memory performance was considerably reduced [50]. Flies with genetically or pharmacologically reduced 5-HT levels in the brain also had a strongly reduced memory formation in a behavioral test wherein flies were trained to avoid a chamber position associated with high temperature [51]. In honey bees, both memory storage and retrieval were reduced when 5-HT was injected prior to conditioning [52-54]. In the desert locust *Schistocerca gregaria* 5-HT and its downstream effector molecules were shown to induce gregarious, swarming behavior [55,56]. Fruit flies with genetically or pharmacologically elevated 5-HT levels showed higher fighting frequencies and more intense fighting than untreated flies [57].

In the periphery, 5-HT seems to affect the performance of certain muscles. It was for example shown to have relaxing properties on the foregut of the migratory locust *Locusta migratoria* [58], while other experiments demonstrated acceleration of the myogenic rhythm of the extensor-tibiae muscle in locusts by 5-HT [59]. Also in migratory locusts, antidiuretic effects were reported for 5-HT, which may result from direct effects on the epithelial and muscular cells of the rectum [60].

5-HT receptors

5-HT is one of the oldest signaling molecules in evolution, which may explain why 5-HT interacts with such a diversity of receptors belonging to the G protein-coupled receptor (GPCR) and the ligand-gated ion-channel families. The first 5-HT receptors are estimated to have appeared 700 to 800 million years ago in single-celled eukaryotes, such as paramecia [61-64]. They may indeed have been amongst the first rhodopsin-like receptors reacting to a chemical [65]. Today, the wealth of 5-HT receptors is generally organized in seven classes, termed 5-HT_{1-7}. The major classes, 5-HT_1, 5-HT_2, and 5-HT_6, possibly evolved from a primordial, ancestral 5-HT receptor over 750 million years ago and are found in diverse phyla from planarians, nematodes, insects to vertebrates, including man [61-63]. The 5-HT_5 and 5-HT_7 receptor classes probably deviated from 5-HT_1 650 to 700 million years ago [61,62]. Since these events predate the estimated divergence of protostomes and deuterostomes about 600 to 650 million years ago [66], the invertebrate and vertebrate serotonergic systems are supposed to own roughly the same main receptor classes [61,67]. However, further gene duplications and structural/functional differentiation led to various subtypes within each main class, and these subtypes are considered to have evolved independently in invertebrates and vertebrates [61,63].

Insect 5-HT receptors are classified based on sequence similarities with their counterparts in vertebrates. Within the same receptor class, the signaling properties seem very well maintained between insects and vertebrates, but their pharmacological characteristics vary significantly. Compared to their mammalian (and other vertebrate) counterparts, relatively few data about pharmacological properties of insect 5-HT receptors are available.

Table 1: The vertebrate 5-HT receptor types, their G protein coupling characteristics and primary responses. Receptor subfamilies with representatives in insects are indicated (•). Abbreviations: AC, adenylyl cyclase; PLC, phospholipase C

Family	G-protein	Response
5-HT_1 •	$G_{i/o}$	AC inhibition
5-HT_2 •	$G_{q/11}$	PLC activation
5-HT_3		Ligand-gated ion channel
5-HT_4	G_s	AC activation
5-HT_5	$G_{i/o}$	AC inhibition
5-HT_6	G_s	AC activation
5-HT_7 •	G_s	AC activation

A recent study provides evidence that agonists of 5-HT receptors may constitute potential lead compounds for new insecticide discovery [68] (see § 6.2).

5-HT receptors in vertebrates

Different classification schemes for 5-HT receptors have been used for more than 50 years. Initially, Gaddum and Picarelli (1957) [69] described D- and M-type receptors, based on pharmacological studies with dibenzyline and morphine. In radio-ligand binding studies on brain homogenates of rat, Peroutka and Snyder (1979) [70] demonstrated the presence of two distinct 5-HT receptor binding sites, named 5-HT_1 and 5-HT_2. These data were amalgamated whereby three main groups of 5-HT receptors were considered, namely 5-HT_1, 5-HT_2 and 5-HT_3 (the latter two corresponding to the D and M receptors, respectively) [71]. This nomenclature still forms the basis for the current classification of 5-HT receptors.

Presently, vertebrate 5-HT receptors are divided into seven main 5-HT receptor families (termed 5-$HT_{1\text{-}7}$) based on their gene organization, sequence similarities, pharmacological properties and downstream signaling pathways [14,64,65,72-74]. Six of these belong to the rhodopsin-like family of GPCRs and the lone exception, 5-HT_3, is a cation-selective chloride-channel that belongs to the ligand-gated ion-channel superfamily. This receptor is composed of five pseudo-symmetrical subunits that surround the central ion-channel. Each subunit contains an extracellular part with a ligand-binding domain, a TM with four α-helices and a cytoplasmatic domain. Activation of the receptor, by binding of 5-HT, leads to the opening of the channel and a quick inward ion current that will cause a depolarization of the plasma membrane [14,65,75]. 5-HT_1 and 5-HT_5 couple to $G_{i/o}$-proteins, which inhibit adenylyl cyclase activity and thus decrease the intracellular cyclic AMP (cAMP) levels. 5-HT_2 couples to $G_{q/11}$-proteins that activate phospholipase C (PLC). PLC hydrolyses membrane bound phosphoinositides, like phosphatidyl inositol 4,5-bisphosphate (PIP_2). This results in the formation of diacylglycerol (DAG) and inositol phosphates like inositol 1,4,5-trisphosphate (IP_3). IP_3 causes Ca^{2+}-exemption in the cell after binding to ligand-regulated Ca^{2+}-channels on the membrane of intracellular Ca^{2+}-storage compartments, such as the endoplasmatic reticulum. DAG remains associated to the cell membrane where it will activate, together with the higher intracellular Ca^{2+}-concentration, Ca^{2+}-dependent protein kinase (PKC). PKC phosphorylates serine and threonine residues of protein substrates, which alters the functional properties of these proteins. 5-HT_4, 5HT_6 and 5-HT_7 couple to G_s-proteins; the $G_{\alpha s}$-subunit stimulates adenylyl cyclase activity and hence increases the intracellular cAMP levels (Table 1) [14,63-65,73,76]. The main receptor families contain different subtypes and isoforms (designated a, b, c …). But even this amount of physical diversity underscores the physiological relevance of 5-HT. An even greater degree of operational diversity can be expected, based on the existence of a great number of splice and RNA editing variants for several 5-HT receptors (see § 5.1.1). In addition, they can be modulated by accessory proteins and chaperones, and can form homo- or heteromers both at the GPCR and the ligand-gated channel level (for a review, see: Hannon and Hoyer, 2008 [65]).

5-HT receptor multiplicity in vertebrates

There are five vertebrate 5-HT_1 receptor subtypes, all encoded by intronless genes: 5-$HT_{1A,B,D,E,F}$ [77-86]. In addition, two polymorphisms have been found to alter the extracellular amino-terminal region of the human 5-HT_{1A} receptor; one is associated with Tourette's syndrome, and one results in loss of response to 5-HT [87,88]. The current 5-HT receptor nomenclature does not contain a 5-HT_{1C} receptor. This receptor was formerly (incorrectly) classified in the 5-HT_1 receptor family, but was renamed 5-HT_{2C} [74]. This 5-HT_{2C} receptor was the first cloned receptor of the 5-HT_2 receptor family [89,90] which now contains three subtypes: 5-$HT_{2A\text{-}C}$ [91-94]. The 5-HT_{2C} receptor forms different isoforms through RNA editing by adenosine deaminases, which affects agonist potency, activation of PLC and selectivity of G protein coupling [95-97]. In mammals, a third family of 5-HT GPCRs comprises at least nine 5-HT_4 isoforms (5-$HT_{4a\text{-}g}$, 5-HT_{4i} and 5-HT_{4n}) resulting from alternative splicing starting from a conserved leucine residue in the intracellular C-terminus. Another variant, 5-HT_{4hb} contains a 14 residue insertion in the second extracellular loop compared to the 5-HT_{4b} variant [98-106]. Moreover, different isoforms that are co-expressed in the same tissue were shown to form dimers [107]. Two subtypes of 5-HT_5 receptors (5-HT_{5A} and 5-HT_{5B}) have been cloned in rodents, sharing 70% overall sequence identity [108-111]. In humans, the 5-HT_{5A} constitutes the only functional 5-HT_5 receptor. The 5-HT_{5B} receptor gene has been mapped

to the human genome, but does not encode a functional protein [112,113]. The first functional 5-HT_6 receptor was cloned from rat cDNA starting from the sequence of the rat histamine H_2 receptor [114]. The rat sequence was later corrected, together with cloning of the human 5-HT_6 receptor sequence [115]. A degenerate PCR approach on human cDNA was used to identify a nonfunctioinal 5-HT_6 receptor splice variant [116]. Members of the 5-HT_7 receptor family were cloned around the same time from rodent and human. Two introns are found in the mammalian receptor encoding gene, from which one is alternatively spliced resulting in differences in the intracellular C-terminus [117-125]. In rat and mouse tissue, three 5-HT_7 isoforms were found, called 5-HT_{7a}, 5-HT_{7b} and 5-HT_{7c}. Two human splice variants correspond to isoforms a and b, but a third constitutes a distinct isoform, called 5-HT_{7d} [118,119,126,127].

5-HT receptors in insects

For many years, the work on 5-HT receptors of invertebrate physiologists and pharmacologists has paralleled that of vertebrate researchers. This resulted in a distinct nomenclature for invertebrate 5-HT receptors that became increasingly confusing when compared to the vertebrate classification. Tierney (2001) [63] proposed a classification system for invertebrate 5-HT receptors based on the vertebrate nomenclature, considering the common ancestry of insect and vertebrate 5-HT receptors. Indeed, as mentioned above, the main receptor classes are believed to have diverged from a primordial 5-HT receptor before the estimated separation of protostomes and deuterostomes [61,62,66].

Based on the conserved amino acid sequences and activated second-messenger systems, insect 5-HT receptors can be classified as 5-HT_1, 5-HT_2 and 5-HT_7 type GPCRs. *D. melanogaster* was the first insect species in which four different 5-HT receptors were pharmacologically characterized, namely a 5-$HT_{1\alpha/A}$, 5-$HT_{1\beta/B}$, 5-$HT_{2\alpha/A}$ and 5-HT_7 receptor [128,129]. In addition, the existence of a second 5-HT_2 receptor (5-$HT_{2\beta/B}$) was suggested and recently confirmed experimentally [41,130,131]. In the field cricket, partial sequences of two 5-HT_1, two 5-HT_2 and a 5-HT_7 receptor were identified as well [132,133]. Two 5-HT_1 receptors were also predicted in the genome of *T. castaneum*, one of these was characterized [134,135] and two 5-HT_1 splice variants were described in *Papilio xuthus* [136]. On the contrary, in the locust *L. migratoria* only one 5-HT_1 receptor was partially cloned and also in *A. mellifera* and *P. americana* only one 5-HT_1 receptor was characterized thus far [137-139]. As in *D. melanogaster* and *G. bimaculatus*, two 5HT_2 receptors were identified from *A. mellifera* [41,128,130-133,140], while in other insects, such as *T. castaneum* and the locust *L. migratoria*, only one 5-HT_2 receptor has been found thus far [134,137]. Lastly, ever since the first insect 5-HT_7 receptor was identified from a *Drosophila* genomic and a cDNA library [129,141], (partial) cDNA sequences of 5-HT_7 receptors from other insects were cloned and some of these were functionally characterized [133,134,137,142,143]. Based on the data from characterized insect 5-HT receptors, most insects seem to possess one 5-HT_7 type serotonin receptor, but may possess two 5-HT_1 and/or two 5-HT_2 receptors.

Similarly to vertebrate receptors, alternative splicing seems a general mechanism for creating extra multiplicity in the insect 5-HT receptor system (for example the 5-HT_1 receptors of *D. melanogaster* and *P. xuthus*) [129,136].

Insect 5-HT receptors often show high expression in nervous tissue (*e.g.* brain and ventral nerve cord) [36,37,133,135,138,142-144]. High 5-HT_1 protein levels in the central nervous system were associated with involvement in visual information processing in honey bee foragers [138] and possible roles in neuroendocrine secretion processes and motility of the gut in cockroaches [139]. The expression of 5-HT_7 in different parts of the honey bee brain suggested a role for the receptor in neural pathways for information processing, learning and memory [142]. Several insect 5-HT_7 receptors were also found to be highly expressed in the salivary gland (FlyAtlas, http://www/flyatlas.cor/) [133,145] and suggested to be involved in 5-HT induced saliva secretion, which was shown to be mediated via an increase in intracellular cAMP in several insect species [44,45,139,146-149]. Immunohistochemistry with antibodies against the *A. aegypti* 5-HT_7 receptor labeled two axons that run in parallel along the hindgut [144]. In *L. migratoria*, high serotonergic immunoreactivity was measured in the midgut and application of 5-HT induced relaxation of the midgut circular muscle [150]. 5-HT_7 receptor-mediated regulation of visceral muscle activity in the gastrointestinal tract may be evolutionary conserved between invertebrates and vertebrates, since also mammalian 5-HT_7 receptors were demonstrated to mediate smooth muscle relaxation of the gastrointestinal tract [151].

Pharmacology of vertebrate 5-HT receptors

The pharmacology of mammalian 5-HT receptor subtypes has been extensively studied (http://www.iuphar-db.org). We will only briefly discuss a selection of agonists and antagonists, focusing on 5-HT_1, 5-HT_2 and 5-HT_7 receptors, since orthologs of these receptor types were found in insects.

5-HT_{1A} receptors are probably the most extensively examined receptor group. One of the first major full and selective 5-HT_{1A} receptor agonists to be discovered was

Table 2. Overview of pharmacological agents tested on insect serotonin receptors

Receptor	Transduction	Agonists	EC_{50}	Antagonists	Cells	References
Drome 5-HT1A CAA77570	decrease cAMP increase IP	5-HT 8-OH-DPAT 5-HT	 1 µM	dihydroergocryptine prazosin d-butaclamol methysergide l-butaclamol yohimbine methiothepin	Cos-7 HEK293T	Saudou *et al*., 1992[129] Gasque *et al*., 2013[41]
Drome 5-HT1B CAA77571	decrease cAMP increase IP	5-HT 8-OH-DPAT 5-HT	 625 nM	dihydroergocryptine d-butaclamol prazosin methysergide yohimbine l-butaclamol methiothepin	Cos-7 HEK293T	Saudou *et al*., 1992[129] Gasque *et al*., 2013[41]
Apime 5-HT1A CBI75449	decrease cAMP	5-HT 5-CT 5-MT	16.9 nM 700.5 nM 3.63 µM	methiothepin prazosin (partial) WAY-100635 (partial)	HEK293	Thamm *et al*., 2010[138]
Trica 5-HT1 KC196076 decrease cAMP	 5-HT	5-HT α-Me5-HT 5-CT 5-MT 8-OH-DPAT 82.7 nM	95 nM 10.7 µM 24.7 µM 91.8 µM 551.0 µM 	prazosin methiothepin methysergide d-butaclamol SB-269970 WAY-100635 *ketanserin (NR)* *mianserin (NR)* *yohimbine (NR)* HEK293	CHO-WTA11 Vleugels *et al*., 2013[135]	Vleugels *et al*., 2013[135]
Peram 5-HT1 FN298392	decrease cAMP	5-HT 5-MT	130 nM (partial)	WAY-100635 (inverse) Methiothepin (neutral)	HEK293	Troppmann *et al*., 2010[139]
Drome 5-HT2(A) CAA57429	?	5-HT N-acetyl-5-HT α-Me5-HT 2-Me5-HT tryptamine 1-Me5-HT 5-MT quipazine 5-MT 8-OH-DPAT 5-CT 5-HT	 99 nM	ritanserin ketanserin pizotifen setoperone spiperone cyproheptadine TFMPP mesulergine methysergide methiothepine rauwolscine buspirone yohimbine bufotenine sulpiride mianserin clozapine cis-flupenthixol haloperidol methiothepin	Cos-1 HEK293T	Colas *et al*., 1995[128] Gasque *et al*., 2013[41]
Drome 5-HT2B CG42796		5-HT	293 nM	methiothepin	HEK293T	Gasque *et al*., 2013[41]
Apime 5-HT2A FR727107	increase Ca2+	5-HT 5-MT 8-OH-DPAT	25.7 nM 70 nM 55.9 µM	SB-269970 mianserin cyproheptadine methiothepin clozapine methysergide	HEK293	Thamm *et al*., 2013[140]
Apime 5-HT2B FR727108	increase Ca2+	5-HT 5-MT 8-OH-DPAT	32.5 nM 60.4 nM 561.5 nM	cyproheptadine ketanserin mianserin clozapine	HEK293	Thamm *et al*., 2013[140]
Drome 5-HT7 M55533	increase cAMP	5-HT 2-Me5HT 5-MT d-LSD	60 nM 0.6 µM 0.8 µM 1.5 µM	dihydroergocryptine d-butaclamol methysergide	NIH 3T3	Witz *et al*., 1990[141]

	increase cAMP	*8-OH-DPAT* 5-HT 8-OH-DPAT 5-HT	*NR* 12 nM	dihydroergocryptine d-butaclamol methysergide prazosin l-butaclamol yohimbine metitepine	Cos-7 HEK293T	Saudou *et al*., 1992[129] Gasque *et al*., 2013[41]
*Apime*5-HT7 CAJ28210	increase cAMP	5-HT 5-CT AS-19 8-OH-DPAT	1.1-1.8 nM 24-40 nM moderate poor	methiothepin (inverse) SB-269970 (poor) *clozapine (NR)*	HEK293	Schlenstedt *et al*., 2006[142]
Trica 5-HT7 XP_966577	increase cAMP	5-HT α-Me5-HT 5-CT 5-MT 8-OH-DPAT 5-HT	27.3 nM 5.2 µM 22.2 µM 30.3 µM 38.8 µM 6.6 nM	ketanserin methysergide methiothepine d-butaclamol prazosin SB-269970 yohimbine WAY-100635 mianserin	CHO-WTA11 HEK293	Vleugels *et al*., 2014[143] Vleugels *et al*., 2014[143]
*Aedae*5-HT7 AAG49292	increase cAMP	5-HT 5-CT 8-OH-DPAT pimozide	39.5 nM moderate moderate poor		CHO-K1	Lee and Pietrantonio, 2003[169]

Pharmacological profiles of heterologously expressed insect 5-HT receptors. Agonists and antagonists are shown according to decreasing potency (per receptor and cell type). Agonists and antagonists that exhibited no response (NR) are shown in grey and italic. Abbreviations used: 5-CT, 5-carboxamidotryptamine; 5-HT, 5-hydroxytryptamine; 5-MT, 5-methoxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; *Aedae*, *Aedes aegypti*; *Apime*, *Apis mellifera*; AS-19, (2S)-N,N-dimethyl-5-(1,3,5-trimethylpyrazol-4-yl)-1,2,3,4-tetrahydronaphthalen-2-amine; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; Cos, fibroblast-like cell line derived from monkey kidney tissue; *Drome*, *Drosophila melanogaster*; EC_{50}, half maximal effective concentration; HEK, human embryonic kidney; IP, inositol phosphate; Me5-HT, methyl hydroxytryptamine; NIH, fibroblast cell line derived from mouse embryos; *Peram*, *Periplaneta americana*; SB-269970, 2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-(2-(4-methyl-1-piperidinyl)ethyl)pyrrolidine; *Trica*, *Tribolium castaneum*; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide.

8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) [84,152]. It was later shown to have some minor affinity for 5-HT_{1D} and 5-HT_7 receptors as well (see IUPHAR database and references therein). Another well-known agonist of 5-HT_1 and 5-HT_7 receptors is 5-CT (5-carboxyamidotryptamine) [117,153,154]. Agonists with minor affinity for 5-HT_1 receptors are 5-MeOT (5-methoxytryptamine) which has high agonistic activity on 5-HT_{2A} and 5-HT_7 receptors, and αm-5-HT (α-methylserotonin) which has higher specificity for 5-HT_2 receptors compared to all other 5-HT receptor (sub)types [117,153,155-157]. Antagonists with very high specificity were found for some mammalian 5-HT receptors. The piperazine drug WAY-100635 is a potent antagonist of 5-HT_{1A} receptors [158,159], while SB-269970 is a potent antagonist of 5-HT_7 receptors [160,161]. However, WAY-100635 was also shown to be a D_4 dopamine receptor agonist [162] and SB-269970 was able to block α2-adrenoceptors to some extent [163]. Several potent 5-HT_2 receptor antagonists with only minor binding affinity for other 5-HT receptors are known, including mianserin, ketanserin, and butaclamol (see IUPHAR database and references therein). However, all have affinity for other biogenic amine receptors as well, with a particularly high binding affinity of ketanserin for α1-adrenergic receptors [164] and of butaclamol for dopamine receptors [165-168]. Several non-selective 5-HT receptor antagonists were characterized, such as methiothepin and methysergide.

Pharmacology of insect 5-HT receptors

Despite the sequence and signaling similarities, the pharmacology of insect 5-HT receptors is curiously different from that of their vertebrate counterparts. The long evolutionary history of the 5-HT signaling systems after the divergence of protostomes and deuterostomes, allowed for further differentiation into subtypes independently in insects and vertebrates [63,64]. During this evolution, selection was likely based on functionally important receptor characteristics, such as ligand binding and G protein coupling, and not the conservation of recognition sites for man-made, synthetic ligands [143].

Although the pharmacology of the mammalian receptors is thoroughly studied (and drugs affecting biogenic amine receptor binding are among the most important in the armory of modern pharmacology), the specificity of these drugs towards insect receptors has never been comprehensively tested and profound characterization studies of insect 5-HT

receptors are scarce. Consequently much less is known about the pharmacology of these receptors. However, it is a recurring fact that the synthetic agonists, designed for vertebrate receptors, are much less potent and often also less efficient than the natural ligand 5-HT on insect 5-HT receptors. For example, the mammalian 5-$HT_{1/7}$ receptor agonists, 8-OH-DPAT and 5-CT, act only as poor agonists on insect 5-$HT_{1/7}$ receptors [135,138,139,142,143,169]. Little information is also available about selective or potent antagonists of insect 5-HT receptors and only limited similarity is observed with the mammalian receptor pharmacology (Table 2). The potent and selective mammalian 5-HT_{1A} antagonist WAY-100635 showed only minor antagonistic activity on insect 5-HT_1 receptors, i.e. from *A. mellifera*, *P. americana* and *T. castaneum* [135,138,139]. The mammalian 5-HT_7 receptor antagonist SB-269970 has been shown to be a potent antagonist of the 5-HT_7 receptor from *C. vicina*, but had only poor influence on activity of the 5-HT_7 receptors from *A. mellifera* and *T. castaneum* [142,143,145]. The non-selective 5-HT antagonists of vertebrate 5-HT receptors, methysergide and methiothepin, were also able to decrease insect 5-HT receptor activity [135,143].

5-HT receptors as pharmacological targets

5-HT receptors as drug targets

Depression and anxiety disorders are two well-known health problems for which compounds that influence 5-HT receptor activity are prescribed. About 80% of all antidepressants on the market are selective serotonin re-uptake inhibitors (SSRIs), preventing 5-HT uptake from the synapse by SERTs. A main disadvantage of SSRIs is that the onset of their therapeutic action requires weeks of treatment. The use of partial agonists of 5-HT_{1A} receptors and inhibitors of 5-HT_{2A} receptors was shown to accelerate clinical antidepressant effects [23,170-173]. Recently, two new antidepressant drugs (vilazodone and vortioxetine) which combine partial 5-HT_{1A} agonist properties with SERT blockade are approved by the FDA (U.S. Food and Drug Administration) to treat depression (Reuters Press Release, 2011; U.S. Food and Drug Administration Press Release, 2013). Partial 5-HT_1 agonists, including certain azapirones, are also used as anxiolytic drugs [172,174]. It is very likely that 5-HT_4 receptor partial agonists could behave as rapid and effective antidepressants as well [175].

The most widely used anti-migraine drugs are triptans which bind with high affinity to 5-$HT_{1B/D/F}$ receptors. However, 5-HT_{1F} is the most attractive target for new anti-migraine drugs since activation of 5-HT_{1B} induces cardiac adverse effects and 5-HT_{1D} agonists – although effective in animal models of migraine – failed to properly attenuate migraine attacks in clinical trials. One selective 5-HT_{1F} agonist (lasmiditan) has successfully completed phase 2 clinical trials (in 2007 for an intravenous and in 2010 for an oral application) [176]. Also agonists of other 5-HT receptors are used in treatment of various health problems (for example [177,178]).

5-HT receptors as potential insecticide targets

The poor affinity and selectivity on insect receptors of the synthetic compounds that were originally designed for mammalian receptor subtypes indicate the importance to look for pharmacological agents that target the insect receptor types more specifically, both for research and insect control. Indeed, although 5-HT receptors are involved in many key processes of insect life, no insecticides that target this receptor group are available at this moment. However, one study provided evidence that 5-HT receptor agonists may form potential lead compounds for new insecticide discovery [68]. They used the 5-HT_{1A} agonist PAPP (1-[(4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine) as a lead compound for new insecticides with novel mode of action. The PAPP scaffold was used to design and synthesize a series of compounds that were evaluated for biological activity against the lepidopteran armyworm *Pseudaletia separata*. Most of the compounds indeed displayed growth-inhibiting potential or induced larval death. Another study in *Drosophila* shows that the general 5-HT receptor antagonist methiothepin inhibits feeding through 5-HT_{2A} [41]. Given the ubiquity of 5-HT signaling in the animal kingdom, any insect control agent targeting 5-HT receptors would have to be thoroughly tested for specificity to avoid toxicity on a wide range of species [55,179].

Concluding remarks

Several interesting studies have highlighted the omnipresent and overwhelming functions of serotonin both in vertebrates as in invertebrates. The number of studies on the role of serotonin in insects is still relatively limited, but these studies already underline the immense importance of serotonin signaling in nearly all major physiological processes in insects.

Identification and classification of insect 5-HT receptors is merely based on sequence similarity with mammalian receptors. Although this information can provide indications for receptor classification and comparison, it does not allow for accurate predictions of the pharmacological characteristics of these receptors. The current pharmacological data available on insect 5-HT receptors suggest that it should be possible to find selective synthetic ligands for specific insect 5-HT receptors. However, a lot of

additional research, contributing both to proper insect 5-HT receptor classification and to understanding their *in vivo* functionality, will be necessary before a selective insecticide can be developed.

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List of abbreviations

5-CT:5-carboxamidotryptamine; 5-HT: 5-hydroxytryptamine or serotonin; 5-HTTP: 5-hydroxytryptohan; 5-MeOT: 5-methoxytryptamine; 8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino) tetralin; αM-5-HT: α-methylserotonin; GPCR: G protein-coupled receptor; IP_3: inositol 1,4,5-trisphosphate; DAG: diacylglycerol; DOPA: 3,4-dihydroxyphenylalanine; LSD: lysergide acid diethylamide; PAPP: 1-[(4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine; PKC: Ca^{2+}-dependent protein kinase; PLC: phospholipase C; PIP_2: phosphatidyl inositol 4,5-bisphosphate; SERT: SERotonin Transporter; SSRI: selective serotonin re-uptake inhibitor; TM: transmembrane segments; TPH: tryptophan hydroxylase.

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